Original Article
Chondroprotective effect of galantamine: a natural alkaloid in osteoarthritis

Qiu-Shi Li1, Xi-Jun Yi1, Tao Yang1, Jing Tian2, Hong-Liang Li1, Yu-Hua Zhao1

1Department of Traumatology, Linyi City People’s Hospital Medical East Region, Linyi 276000, Shandong, China; 2Linyi Cancer Hospital Operating Room, Linyi 276000, Shandong, China

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Abstract: Present investigation evaluates the chondroprotective effect of galantamine in monosodium iodoacetate induced osteoarthritis rats. Osteoarthritis was induced by monosodium iodoacetate injection (3 mg in 50 µl volume) in the space of knee joint. Osteoarthritis rats were treated with galantamine (5 & 10 mg/kg, im) for the duration of 14 days. Chondroprotective effect of galantamine was assessed by observation of knee joint (swelling & limping), roentgenographical and histopathological study. Serum cytokine and protein expression of matrix metalloproteinase (MMPs), cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS) were also estimated in monosodium iodoacetate induced osteoarthritis rats. The result of the given study suggested that galantamine significantly decreases (P<0.01) the swelling and limping in osteoarthritis rats. Moreover roentgenographical and histopathological study reported that galantamine attenuated the degenerative change in the knee joint of osteoarthritis rats. There were significant (P<0.01) decrease in the protein expression of MMP2, MMP9, COX2 & iNOS in galantamine treated group of rats compared to osteoarthritis rats. Serum concentration of tumor necrosis factor α (TNFα), Interleukin 1β (IL1β) & 10 (IL10) also significantly decreases in galantamine treated group compared osteoarthritis rats. Present study concluded that galantamine possess chondroprotective effect in osteoarthritis by reducing the serum cytokine level and protein expressions of MMP2, MMP9, COX2 & iNOS in monosodium iodoacetate induced osteoarthritis rats.

Keywords: Galantamine, osteoarthritis, chondroprotective, cytokines, matrix metalloproteinase

Introduction

Osteoarthritis is a chronic joint disorder characterized by degeneration of cartilages, joint pain and stiffness. Previous study reported that degeneration of cartilages resulted due to imbalance in its synthesis and degenerative pathways [1]. Pathogenesis of osteoarthritis reveals that tissue proteinases enhances the breakdown of cartilages and inflammatory mediators like interleukin-1β and tumor necrosis factor α upregulated its expressions. Moreover, interleukin-1β alters the metabolic activities in chondrocytes and there by downregulates the generation of cartilage matrix [2]. Non steroidal anti-inflammatory drugs (NSAIDs) used in inflammation associated with osteoarthritis but long term application induces gastric ulceration [3].

In osteoarthritis, inflammatory mediators enhances the production of reactive oxygen species which is also alters the homeostasis of cartilage [4]. Increased oxidative stress in chondrocytes damages mitochondria and thereby increases the production of lipid peroxidation decrease in the superoxide dismutase. It also proves the role of antioxidant in the management of osteoarthritis [5, 6]. All these factors plays important role in the development of osteoarthritis and hence it is difficult to achieved complete therapeutic effect by inhibiting one or two mediators of inflammation.

Literature suggested that cholinergic system plays a vital role in the development of osteoarthritis [7]. In synovial fluid the concentration of Ach controls the cytokines production in the joint by down regulating the expression of α7nAChR protein and there by possess anti inflammatory effect [8]. Ach concentration is regulated by acetylcholinesterase as it metabolizes the Ach in to acetate and choline. Thus
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acetylcholinesterase inhibitor may play a role in the management of osteoarthritis [9, 10].

The use of herbal drugs in the management of chronic disorders enhances, as it decreases the chances of adverse drug reaction. Many natural products possess the multifunctional effect and thereby possess beneficial effect for the management of chronic disorders like osteoarthritis [11]. Galantamine is a natural alkaloid clinically used in the management of alzheimers [12]. It also possesses acetylcholine esterase inhibitor and antioxidant effect [13, 14]. Galantamine reported to control the homeostasis of glucose, thereby lower the glycosylated hemoglobin in the patient suffering from diabetes [15]. On the basis of these properties present study deals with the chondroprotective effect of galantamine in osteoarthritis.

Material and method

Animals

Healthy male Wistar rats (200-250 g) were procured from Avian Disease Research Center, Sichuan Agricultural University, China. All the animals were kept under controlled condition as per the guidelines with pallet feed and water ad libitum. Investigation protocols of the present study approved by Institutional ethical committee.

Induction of osteoarthritis

Healthy Wistar rats were anesthetized with phenobarbital and osteoarthritis was induced by a injection of monosodium iodoacetate (3 mg in 50 µl volume) into intra articular space of the knee. Moreover, control group of rats were injected with equal volume of saline solution. Galantamine in two different doses (5 & 10 mg/kg, i.m.) were given to respective treatment group for the 14 days after the injection of monosodium iodoacetate. During the protocol rats were weighed and assessment of swelling at knee. Severity of swelling categorized as severe, mild and no change [16].

Roentgenographical and histological analysis

14 days after the monosodium iodoacetate injection, all the rats were analyzed for changes in the morphology of articular structure of knee bone, development of osteophytes, erosion in cartilages and loss at joint region [17]. Later, Knee joint of all the rats were isolated and added to formalin (10%) thereafter 10% formic acid used for the decalcification of knee joint. Tissue sections were added to paraffin and stained by H&E and SOFG staining [16].

Estimation of activity of MMP2 & MMP9

Articular cartilages of rats were harvested 14th day of monosodium iodoacetate injection. Gelatinase enzymes activities were estimated by gelatin zymography. Zymogram gels (10%) were used for electrophoresis of proteins extracted through cartilage tissues. Densitometric analysis was done for the estimation of each band area [18].

Estimation of serum cytokine

Enzyme linked immunosorbent assay was used for the estimation of cytokine level such as tumor necrosis factor α, Interleukin 1β & 10 in the serum [19].

Estimation COX2 and iNOS

Cyclooxygenase 2 (COX 2) and inducible nitric oxide synthase (iNOS) level was estimated from whole protein. Quantitative estimation was achieved through densometric determination of bands by Image Quant software [19].

Statistical analysis

All the values of these experiments were articulated as mean ± SD and the data was statistically analyzed by one-way ANOVA and thereafter applied to Dunnett post hoc test. P<0.05 was considered significant statistically.

Result

Assessment of swelling and limping

Table 1 Showed the effect of galantamine on swelling and limping through observation in monosodium iodoacetate induced osteoarthritis rats. It was observed that monosodium iodoacetate induces osteoarthritis rats resulted in swelling in joints and difficulty in walking (Limping). Treatment with galantamine significantly (P<0.01) reduces the score of swelling and limping as compared to negative control group of rats. This decrease in swelling and limping score with galantamine treatment was found to be dose dependent.

Roentgenographical and histopathological estimation

After 14 days of treatment rats were examine for rotenographic analysis. It was observed that monosodium iodoacetate injection develops
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Table 1. Effect of galantamine on swelling and limping through observation in monosodium iodoacetate induced osteoarthritis rat

<table>
<thead>
<tr>
<th>Observations</th>
<th>Control</th>
<th>Negative control</th>
<th>Galantamine 5 mg/kg</th>
<th>Galantamine 10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swelling</td>
<td>Sever</td>
<td>2</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Average Score</td>
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<td>1.8±0.42*</td>
<td>0.9±0.73**</td>
<td>0.7±0.67**</td>
</tr>
<tr>
<td>Limping</td>
<td>Sever</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Average Score</td>
<td>0</td>
<td>1.4±0.16**</td>
<td>0.7±0.22**</td>
<td>0.3±0.15**</td>
</tr>
</tbody>
</table>

Values are means ± SD (n=10); **P<0.01 compared to control group, ***P<0.01 compared to Negative control group.

Figure 1. Roentgenographically and histopathologically estimation of galantamine’s effect on knee joint lesions in monosodium iodoacetate induced osteoarthritis rat (H&E staining and SOFG staining, 100×) (n=10). A: Control group; B: Negative control group; C: Galantamine 5 mg/kg; D: Galantamine 10 mg/kg.

the degenerative changes in the joints like rough edge surface on cartilages and altered patellar displacement tendency. Whereas, these change were significantly reduced in the galantamine treated group of compared to negative control group of rats as shown in Figure 1A. Moreover the Histopathology study by H&E staining suggested that irregular surface associated with degeneration of cartilages and development of ulcer in negative control group. These changes significantly attenuated by galantamine treatment as shown in Figure 1B.

Staining with SOFG concealed that depletion of PD in the cartilage tissues of negative control group of rats. This depletion of PD was ameliorated by galantamine treatment as shown in Figure 1C.

Estimation of galantamine effect on the activity of matrix metalloproteinase 2 & 9 by gelatinase assay

Effect of galantamine on the activity of MMP2 and MMP9 in monosodium iodoacetate indu-
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The activity of MMP2 & MMP9 was found to be significantly increased (P<0.01) in monosodium iodoacetate injected rats compared to normal rats. Whereas, galantamine treatment significantly (P<0.05, P<0.01) reduces the activity of MMP2 & MMP9 proteins.

Estimation of serum cytokines level

In osteoarthritis serum cytokine level found to be increased compared to normal level of it. Serum concentration of TNFα, IL1β & IL10 were found to be significantly increased up to 92.7,

98.4 & 70.5 pg/ml respectively in negative control group of rats. However, galantamine treatment significantly decreases (P<0.05, P<0.01) the serum concentration of TNFα & IL1β compared to negative control group of rats as shown in Table 2. Moreover the concentration IL10 was found to be increased in galantamine treated group compared to negative control group of rats.

Estimation of COX-2 and iNOS protein expression

Protein expression of COX2 and iNOS found to be increased in negative control group of rats compared to control group of rats. There were significant (P<0.05, P<0.01) decrease in the galantamine treated group compared to negative control group of rats. This decrease in the protein expression of COX2 and iNOS was found to be dose dependent as shown in Figure 3. These alteration in protein expression confirms the role of galantamine in the management of osteoarthritis.

Discussion

Osteoarthritis means chronic degeneration of cartilages...
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Histopathology study was achieved by H&E and SOFG staining of knee joint. Histopathology study reveals that treatment galantamine significantly attenuates the cartilage degeneration induced by monosodium iodoacetate in rats.

Previous studies suggested that degeneration of extracellular matrix controlled by the MMPs and its subclasses viz. MMP2 and MMP9 degrades elastin and gelatin like proteins [21]. Protein expression of MMP2 and MMP9 found to be increased in the synovial fluid of patients suffering from osteoarthritis and a study suggested a MMP9 is directly related to destruction of joints [22]. Thus the drug decreases the expression of these MMPs effectively manages the osteoarthritis [23]. In the given study galantamine significantly (P<0.05, P<0.01) decreases the expression of MMP2 and MMP9 and thereby manages the osteoarthritis.

The level of cytokines, inflammatory mediators and iNOS was also increases in the osteoarthritis and there by damages the chondrocytes [24]. NSAIDs improve the clinical condition of patient suffering from osteoarthritis by reducing the COX2 level in the synovial joints and TNFα & IL1β in the serum of osteoarthritis rats.

Moreover, iNOS increases the oxidative stress and lead to destruction of cartilages in the joint [25]. Galantamine improves the management of osteoarthritis by decreasing the protein expression of COX2 and iNOS in the monosodium iodoacetate induced osteoarthritis rats.

Conclusion

The present investigation concludes that galantamine possesses chondroprotective effect in monosodium iodoacetate induced osteoarthritis rats by reducing expression of different proteins like MMP2, MMP9, TNFα, IL1β, COX2 and iNOS expression.

Disclosure of conflict of interest

None.

References


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